

WE CLAIM:

1. A gene expression modulation system comprising:
 - a) a first gene expression cassette that is capable of being expressed in a host cell comprising a polynucleotide encoding a first polypeptide comprising:
 - i) a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated;
 - ii) a ligand binding domain comprising a ligand binding domain from a nuclear receptor;
 - b) a second gene expression cassette that is capable of being expressed in the host cell comprising a polynucleotide encoding a second polypeptide comprising:
 - i) a transactivation domain; and
 - ii) a ligand binding domain comprising a ligand binding domain from a nuclear receptor other than ultraspiracle (USP).
- 15 2. The gene expression modulation system according to claim 1, further comprising a third gene expression cassette comprising:
 - i) a response element to which the DNA-binding domain of the first polypeptide binds;
 - ii) a promoter that is activated by the transactivation domain of the second polypeptide; and
 - iii) the gene whose expression is to be modulated.
- 20 3. The gene expression modulation system according to claim 1, wherein the ligand binding domain of the first polypeptide is an ecdysone receptor polypeptide.
4. The gene expression modulation system according to claim 1, wherein the ligand binding domain of the second polypeptide is a retinoid X receptor polypeptide.
- 25 5. A gene expression modulation system comprising:
 - a) a first gene expression cassette that is capable of being expressed in a host cell comprising a polynucleotide encoding a first polypeptide comprising:
 - i) a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated; and
 - ii) a ligand binding domain comprising a ligand binding domain from an ecdysone receptor; and
 - b) a second gene expression cassette that is capable of being expressed in the host cell comprising a polynucleotide encoding a second polypeptide

comprising:

- i) a transactivation domain; and
- ii) a ligand binding domain comprising a ligand binding domain from a retinoid X receptor.

5 6. The gene expression modulation system according to claim 5, further

comprising a third gene expression cassette comprising:

- i) a response element to which the DNA-binding domain of the first polypeptide binds;
- ii) a promoter that is activated by the transactivation domain of the second polypeptide; and
- iii) the gene whose expression is to be modulated.

10 7. The gene expression modulation system according to claim 5, wherein the ligand binding domain of the first polypeptide is encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10.

15 8. The gene expression modulation system according to claim 5, wherein the ligand binding domain of the first polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

20 9. The gene expression modulation system according to claim 5, wherein the ligand binding domain of the second polypeptide is encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, and SEQ ID NO: 30.

25 10. The gene expression modulation system according to claim 5, wherein the ligand binding domain of the second polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, and SEQ ID NO: 40.

30 11. A gene expression modulation system comprising:

- a) a first gene expression cassette that is capable of being expressed in a host cell comprising a polynucleotide encoding a first polypeptide comprising:

- i) a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated; and
 - ii) a ligand binding domain comprising a ligand binding domain from a retinoid X receptor; and
- 5 b) a second gene expression cassette that is capable of being expressed in the host cell comprising a polynucleotide encoding a second polypeptide comprising:

- i) a transactivation domain; and
- ii) a ligand binding domain comprising a ligand binding domain from an ecdysone receptor.

10 12. The gene expression modulation system according to claim 11, further comprising a third gene expression cassette comprising:

- i) a response element to which the DNA-binding domain of the first polypeptide binds;
- ii) a promoter that is activated by the transactivation domain of the second polypeptide; and
- iii) the gene whose expression is to be modulated.

15 13. The gene expression modulation system according to claim 11, wherein the ligand binding domain of the first polypeptide is encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, 20 SEQ ID NO: 28, SEQ ID NO: 29, and SEQ ID NO: 30.

25 14. The gene expression modulation system according to claim 11, wherein the ligand binding domain of the first polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, and SEQ ID NO: 40.

30 15. The gene expression modulation system according to claim 11, wherein the ligand binding domain of the second polypeptide is encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10.

16. The gene expression modulation system according to claim 11, wherein the ligand binding domain of the second polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO:

DRAFT - 02/2020

14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and
SEQ ID NO: 20.

17. A gene expression cassette comprising a polynucleotide encoding a hybrid
polypeptide comprising a DNA-binding domain or a transactivation domain, and an ecdysone
5 receptor ligand binding domain, wherein the DNA binding domain or the transactivation
domain is from a nuclear receptor other than an ecdysone receptor.

18. A gene expression cassette comprising a polynucleotide encoding a hybrid
polypeptide comprising a DNA-binding domain or a transactivation domain, and a retinoid X
receptor ligand binding domain, wherein the DNA binding domain or the transactivation
10 domain is from a nuclear receptor other than a retinoid X receptor.

19. A gene expression cassette comprising a polynucleotide encoding a hybrid
polypeptide comprising either a) a DNA-binding domain encoded by a polynucleotide
comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 41,
SEQ ID NO: 43, and SEQ ID NO: 45, or b) a transactivation domain encoded by a
15 polynucleotide comprising a nucleic acid sequence selected from the group consisting of
SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, and SEQ ID NO: 53, and a ligand binding
domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the
group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID
NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ
20 ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO:
26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, and SEQ ID NO: 30.

20. A gene expression cassette comprising a polynucleotide encoding a hybrid
polypeptide comprising either a) a DNA-binding domain comprising an amino acid sequence
selected from the group consisting of SEQ ID NO: 42, SEQ ID NO: 44, and SEQ ID NO: 46,
25 or b) a transactivation domain comprising an amino acid sequence selected from the group
consisting of SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, and SEQ ID NO: 54, and a
ligand binding domain comprising an amino acid sequence selected from the group consisting
of SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ
ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO:
30 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36,
SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, and SEQ ID NO: 40.

21. An isolated polynucleotide encoding an ecdysone receptor polypeptide or a
retinoid X receptor polypeptide comprising a truncation mutation, wherein the truncation
mutation reduces ligand binding activity of the ecdysone receptor polypeptide or the retinoid

X receptor polypeptide.

22. An isolated polynucleotide encoding an ecdysone receptor polypeptide or a retinoid X receptor polypeptide comprising a truncation mutation, wherein the truncation mutation enhances ligand binding activity of the ecdysone receptor polypeptide or the 5 retinoid X receptor polypeptide.

23. An isolated polynucleotide encoding a retinoid X receptor polypeptide comprising a truncation mutation, wherein the truncation mutation increases ligand sensitivity of the retinoid X receptor polypeptide.

24. An isolated polynucleotide encoding a retinoid X receptor polypeptide 10 comprising a truncation mutation, wherein the truncation mutation increases ligand sensitivity of a heterodimer, wherein the heterodimer comprises said retinoid X receptor polypeptide and a dimerization partner.

25. The isolated polynucleotide according to claim 24, wherein the dimerization partner is an ecdysone receptor polypeptide.

15 26. An isolated polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, and SEQ ID NO: 30.

20 27. An isolated polypeptide encoded by the isolated polynucleotide according to claim 26.

28. An isolated truncated ecdysone receptor polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 25 18, SEQ ID NO: 19, and SEQ ID NO: 20.

29. An isolated truncated retinoid X receptor polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, and SEQ ID NO: 40.

30 30. A method of modulating the expression of a gene in a host cell comprising the

gene to be modulated comprising the steps of:

- a) introducing into the host cell the gene expression modulation system according to claim 1; and

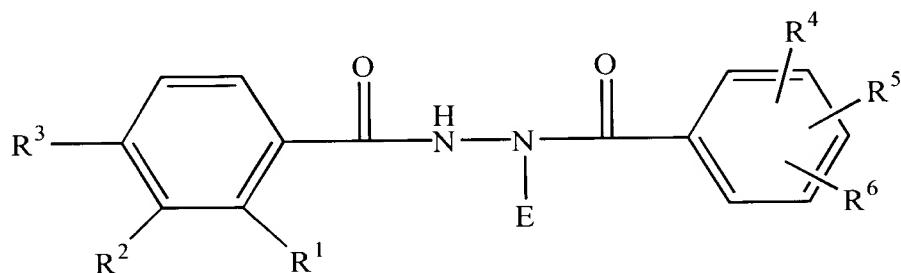
P0002610 "DRAFT" 02/2020

b) introducing into the host cell a ligand that independently combines with the ligand binding domains of the first polypeptide and the second polypeptide; wherein the gene to be expressed is a component of a chimeric gene comprising:

- 5 i) a response element to which the DNA binding domain from the first polypeptide binds;
- ii) a promoter that is activated by the transactivation domain of the second polypeptide; and
- iii) a gene whose expression is to be modulated,

whereby expression of the gene is modulated in the host cell.

10 31. The method according to claim 30, wherein the ligand is either
a) a compound of the formula:



wherein:

- E is a (C₄-C₆)alkyl containing a tertiary carbon or a cyano(C₃-C₅)alkyl containing a
15 tertiary carbon;
R¹ is H, Me, Et, i-Pr, F, formyl, CF₃, CHF₂, CHCl₂, CH₂F, CH₂Cl, CH₂OH, CH₂OMe,
CH₂CN, CN, C°CH, 1-propynyl, 2-propynyl, vinyl, OH, OMe, OEt, cyclopropyl,
CF₂CF₃, CH=CHCN, allyl, azido, SCN, or SCHF₂;
R² is H, Me, Et, n-Pr, i-Pr, formyl, CF₃, CHF₂, CHCl₂, CH₂F, CH₂Cl, CH₂OH, CH₂OMe,
16 CH₂CN, CN, C°CH, 1-propynyl, 2-propynyl, vinyl, Ac, F, Cl, OH, OMe, OEt, O-n-
Pr, OAc, NMe₂, NEt₂, SMe, SET, SOCF₃, OCF₂CF₂H, COEt, cyclopropyl, CF₂CF₃,
CH=CHCN, allyl, azido, OCF₃, OCHF₂, O-i-Pr, SCN, SCHF₂, SOMe, NH-CN, or
joined with R³ and the phenyl carbons to which R² and R³ are attached to form an
ethyleneedioxy, a dihydrofuryl ring with the oxygen adjacent to a phenyl carbon, or a
20 dihydropyryl ring with the oxygen adjacent to a phenyl carbon;
R³ is H, Et, or joined with R² and the phenyl carbons to which R² and R³ are attached to
form an ethylenedioxy, a dihydrofuryl ring with the oxygen adjacent to a phenyl
carbon, or a dihydropyryl ring with the oxygen adjacent to a phenyl carbon;
25 R⁴, R⁵, and R⁶ are independently H, Me, Et, F, Cl, Br, formyl, CF₃, CHF₂, CHCl₂, CH₂F,

CH₂Cl, CH₂OH, CN, C°CH, 1-propynyl, 2-propynyl, vinyl, OMe, OEt, SMe, or SEt,
or

- b) an ecdysone, 20-hydroxyecdysone, ponasterone A, muristerone A, an
oxysterol, a 22(R) hydroxycholesterol, 24(S) hydroxycholesterol, 25-
5 epoxycholesterol, T0901317, 5-alpha-6-alpha-epoxycholesterol-3-sulfate (ECHS), 7-
ketcholesterol-3-sulfate, farnesol, a bile acid, a 1,1-biphosphonate ester, or Juvenile
hormone III.

32. The method according to claim 31, wherein the method further comprises a
second ligand, wherein the second ligand is 9-cis-retinoic acid or a synthetic analog of
10 retinoic acid.

33. An isolated host cell into which the gene expression modulation system
according to claim 1 has been introduced.

34. The isolated host cell according to claim 33, wherein the host cell is selected
from the group consisting of a bacterial cell, a fungal cell, a yeast cell, a nematode cell, an
15 insect cell, a fish cell, a plant cell, an avian cell, an animal cell, and a mammalian cell.

35. A non-human organism comprising a host cell into which the gene
expression
modulation system according to claim 1 has been introduced.

36. The non-human organism according to claim 35, wherein the non-human
20 organism is selected from the group consisting of a bacterium, a fungus, a yeast, a nematode,
an insect, a fish, a plant, a bird, an animal, and a mammal.